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Preview Notes

Molecular monitoring of Staphylococcus aureus in chronic lesions: a descriptive study

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ABSTRACT

The presence of infection in the wound delays the healing process. The main isolated micro-organisms are Staphylococcus aureus and Pseudomonas aeruginosa. **Aim:** To analyze the phenotypic and genotypic profile of Staphylococcus aureus strains in chronic lesions of outpatients treated with 2% hydrogel or polyurethane. Such analysis will support the decision with regard to the appropriate treatment and ensure greater speed in the tissue repair process. **Method:** this is a descriptive study that uses a quantitative approach. It is carried out through clinical specimen collection of lesions by swab, involving bacterial culture, identification and molecular characterization.

Descriptors: Ulcer; Wound Infection; Staphylococcus Aureus; Nursing.

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Problem situation and significance

Staphylococcus aureus (S. aureus) one of the most prevalent microorganisms in lesions[1]. However, the identification of the etiologic agent solely through the use of clinical signs and symptoms is difficult due to the large number of micro-organisms that can be isolated in culture[1]. The use of phenotypic methods, and especially genotypic ones, to determine the microbial presence and to evaluate the antimicrobial susceptibility profile and the virulence of genes in bacteria present in chronic wounds, can guide the appropriate treatment.

Hypothesis

The Staphylococcus aureus present in chronic wounds have presented a range of different antimicrobial resistance profiles.

AIMS

Overall objective: to analyze the phenotypic and genotypic profile of S. aureus strains in chronic lesions of outpatients treated with 2% hydrogel or polyurethane board. Specific objectives: to identify strains of S. aureus in chronic lesions using phenotypic and genotypic methods; to determine the susceptibility of these microorganisms to commonly used antimicrobials and biocides; to detect the presence of the mecA and pyl genes using PCR (polymerase chain reaction); to verify the genetic diversity of the strains detected through PFGE (pulsed-field gel electrophoresis); to evaluate the influence of 2% hydrogel or polyurethane in lesions of patients colonized, contaminated or infected with S. aureus.

METHOD

This is a descriptive study that uses a quantitative approach. It was carried out in the Wound Healing Clinic of the University Hospital Antônio Pedro (UHPH/FFU) and the Engenhoca Community Polyclinic. The population treated at the Clinic consists of an average of 186 patients per year[2]. The sample was determined by convenience, with 70 patients or four months of collection (November 2014 to February 2015), whichever was reached first. Inclusion criteria: aged over 18 years; presenting chronic tissue damage; using 2% hydrogel or polyurethane in the lesion. Exclusion criteria: presenting chronic lesion(s) with an area smaller than 3cm2 or only necrosis in bed; using immunosuppressive drugs. Discontinuity criteria: product changes during the 15 days between collection of the first and the second swab.

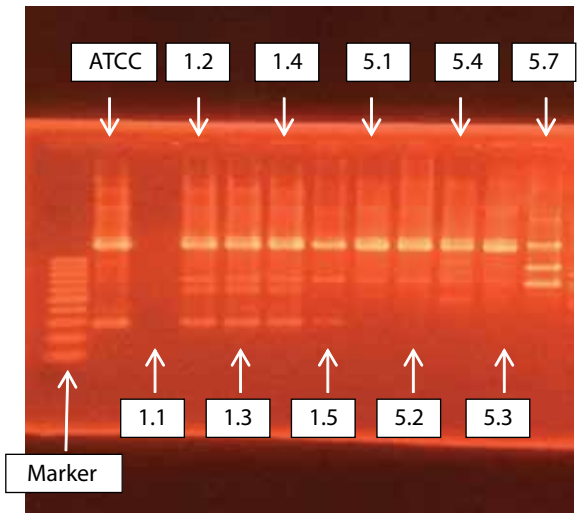
Data collection was obtained in two nursing visits (D0 and D15) involving records of patients: identification, clinical, injury description, wound area calculation using planimetry and photography. The microbiological analysis will be performed in the Microbiological Control Laboratory (MCL) of the Faculty of Pharmacy and will take place through the sowing of the swab in Mannitol Salt (DIFCO). After incubation, suggestive colonies of S. aureus will be submitted to the Gram stain test, catalase, coagulase, disk diffusion tests and assessments in terms of the determination of minimum inhibitory concentration according to the Clinical and Laboratory Standards Institute (CLSI).

Gene evaluation will occur by means of PCR. The amplicons will be subjected to electrophoresis on 1.5% agarose gel, stained with ethidium bromide and visualized under ultraviolet light. To evaluate the clonal diversity of the strains found, the PFGE will be held using the CHEF-DR III system.

Statistical analysis will be done in stages. First stage: tabulation of clinical data from the wounds in spreadsheets in Microsoft Excel software (Serial Number KGFVY-7733B-8WCK9-KTG64-BC7D8); assessment of normality by the Shapiro-Wilk test (sample smaller than 50 subjects) or Kolmogorov-Smirnov (sample greater than 50 subjects) and analysis by descriptive statistics in the BioStat 5.3 software (free license). Second stage: correlations of microbial load using Pearson (normal data) or Spearman (non-normal data) coefficients; antimicrobial resistance; determining genes associated with this resistance at a significance level of 0.05.

To assess whether different strains of S. aureus infect or colonize the same wound, there was a pretest with polymorphic amplification assays DNA-PCR (RAPD-PCR) using primer 1254 (5'-CC-GCAGCCAA-3 ') from different colonies from the bacterial isolation and from the same patient. The products were analyzed by electrophoretic run on agarose gel at 1.5% (70V for 2 hours) for two samples determined by convenience (five different colonies obtained from the primary isolation of two patients - Figure 1). Through this analysis, we observed that 75% of the isolated colonies of patient 1 showed the same genetic profile, suggesting that this is a single clone. In colony 1.1 there was no amplification, probably due to failure in obtaining a DNA from the sample. In patient 5, the genetic profiles were similar in 60% of the cases. Thus, it is believed that the isolation and identification of only a single colony is representative of the population of S. aureus of the lesion.

Picture 1: Electrophoretic Run Results. Niterói, 2015.



Subject 1.2014 1.2014
(Colonies: 1.1; 1.2; 1.3; 1.4; 1.5)
Subject 1.2014 5.2014
(Colonies: 5.1; 5.2; 5.4; 5.3; 5.7)

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